Calcium requirements: new estimations for men and women by cross-sectional statistical analyses of calcium balance data from metabolic studies[1–4]

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ABSTRACT

Background: Low intakes of calcium are associated with an increased risk of both osteoporosis and cardiovascular disease.

Objective: To provide new estimates of the average calcium requirement for men and women, we determined the dietary calcium intake required to maintain neutral calcium balance.

Design: Calcium balance data [calcium intake – (fecal calcium + urinary calcium)] were collected from 155 subjects [women: $n = 73$; weight: 77.1 ± 18.5 kg; age: 47.0 ± 18.5 y (range: 20–75 y); men: $n = 82$; weight: 76.6 ± 12.5 kg; age: 28.2 ± 7.7 y (range: 19–64 y)] who participated in 19 feeding studies conducted in a metabolic unit. Balance data from the final 6–12 d of each dietary period (minimum length:18 d) of each study (1–9 observations per subject) were analyzed. Data were excluded if individual intakes of magnesium, copper, iron, phosphorus, or zinc fell below the estimated average requirements or exceeded the 99th percentile of usual intakes from the 1994 Continuing Survey of Food Intakes by Individuals (for iron, above the upper limit). Daily intakes of calcium ranged between 415 and 1740 mg. The relation between intake and output was examined by fitting random coefficient models. Coefficients were included to test for sex and age differences.

Results: The models predicted a neutral calcium balance [defined as calcium output (Y) equal to calcium intake (C)] at intakes of 741 mg/d [95% prediction interval (PI): 507, 1035; $Y = 148.29 + 0.80C$], $9.4 \text{ mg} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$ [95% PI: 6.4, 12.9; $Y = 1.44 + 0.85C$], or $0.28 \text{ mg} \cdot \text{kcal}^{-1} \cdot \text{d}^{-1}$ [95% PI: 0.19, 0.38; $Y = 0.051 + 0.816C$]. Neither age nor sex affected the estimates when calcium intakes were expressed as mg/d or as $\text{mg} \cdot \text{kg body wt}^{-1} \cdot \text{d}^{-1}$.

Conclusion: The findings suggest that the calcium requirement for men and women is lower than previously estimated. *Am J Clin Nutr* 2007;86:1054–63.

KEY WORDS Calcium intake, calcium excretion, calcium balance, calcium requirement

INTRODUCTION

Calcium has chemical properties indispensable for skeletal function such that adequate dietary calcium intake is required to achieve full accretion of bone mass prescribed by genetic potential. On the other hand, it is well recognized that calcium has a limited role in maintaining bone health because calcium adequacy alone does not fully protect against bone loss (1), especially that associated with age and menopause (2). It has not been firmly established that increasing calcium intake to current recommendations affords a significant reduction in fracture incidence (3), the only sequela of importance in osteoporosis.

Adequate Intakes (AIs), rather than estimated average requirements (EARs), were set for calcium on the basis of several concerns, including uncertainties in the precision and significance of the balance studies needed to determine a desirable retention model and the lack of concordance between mean calcium intakes and experimentally derived values predicted to achieve a desirable level of calcium retention (4). Within this context of uncertainty of the calcium requirement, only 1 in 4 Americans meets their AI for calcium. Women are even less likely than are men to have calcium intakes above their AI (5). For example, only 5% of women aged 51–70 y exceed the AI for calcium for that sex-age group (1200 mg/d; 4). Instead, their estimated mean calcium intake during the 2001–2002 National Health and Nutrition Examination Survey was 701 mg/d.

Constraints on calcium in maintaining skeletal function becomes problematic when functional outcomes related to bone health are used to establish dietary calcium intake guidelines. Because insufficient data exist to establish an EAR for calcium and because the calcium intakes of American men and women (from adolescence to old age) have failed to meet the AI (5), further research is warranted to set an EAR for the mineral. Current AIs for calcium rely solely or heavily on data from calcium balance studies (4). In particular, calcium intakes around predicted zero balance are needed to model the precise relation between mineral intake and loss and retention near zero balance

(6). A larger base of quantitative data for calcium would enhance the ability to estimate the average calcium requirement and subsequently augment the development of sound recommendations for calcium intakes, particularly for older age groups.

A series of tightly controlled metabolic in-house feeding studies conducted between 1976 and 1995 at the US Department of Agriculture, Agricultural Research Service Grand Forks Human Nutrition Research Center (Grand Forks, ND) provided a broad range of calcium intake and output data from healthy individuals. These data were used to estimate the amount of dietary calcium needed to maintain zero calcium balance, information that is useful in establishing an EAR for calcium.

SUBJECTS AND METHODS

Forty-two tightly controlled metabolic in-house feeding studies were conducted between 1976 and 1995 at the US Department of Agriculture, Agricultural Research Service Grand Forks Human Nutrition Research Center to determine specific outcomes of nutritional challenges (eg, marginal zinc deficiency) on physiologic function. Nineteen of those studies (**Table 1**; 7–22) measured calcium balance and incorporated design components, such as a control (nutritionally replete) dietary period, which is relevant for estimating the calcium requirement by cross-sectional statistical analysis. Foods were the only source of calcium in these studies; no calcium supplements were given. Common design characteristics of the 19 individual original metabolic studies used in the present study are summarized below. Details about each study are available in the references cited in Table 1.

Subjects

Each of the original metabolic studies (Table 1) was reviewed and approved separately by the University of North Dakota Protection of Human Subjects Committee (studies 1-4) or by the University of North Dakota Institutional Review Board (studies 5–19 and the current study). Subjects were informed verbally and in writing about the purpose and design for each original metabolic study, and they provided written informed consent to participate in protocols that followed the guidelines of the Declaration of Helsinki regarding the use of human subjects.

Recruitment

In all the studies, healthy women and men were recruited by public advertisement. They were selected to enter each study after being informed in detail of the nature of the research, including the risks and benefits.

Inclusion criteria

Healthy subjects were screened on site and were selected on the basis of medical data (no evidence of alcoholism; normal bone, kidney, thyroid, and liver function; normal blood pressure and fasting glucose; no chronic medication use; and negative tuberculosis result on a lung scan), psychological history [free of psychopathology as determined by the Minnesota Multiphasic Personality Inventory (NCS Assessment, Minneapolis, MN) and by an extensive in-house psychological history questionnaire and clinical interview], and diet history (no pertinent food

allergies or refusal to eat required foods). Postmenopausal women agreed to discontinue hormone replacement therapy before joining a specific study.

Living environment

Accepted female subjects [$n = 73$; weight ($\bar{x} \pm SD$): 77.1 ± 18.5 kg; age: 47.0 ± 18.5 y (range: 20–75 y)] and male subjects [$n = 82$; weight: 76.6 ± 12.5 kg; age: 28.2 ± 7.7 y (range: 19–64 y)] resided for the entire length (typically 6 mo) of the individual studies in the metabolic ward at the Grand Forks Human Nutrition Research Center. Subject ethnicity was predominantly white ($n = 144$), with additional participation by blacks ($n = 3$), American Indians or Alaskan natives ($n = 3$), Asians ($n = 2$), and Hispanics ($n = 2$); 1 person was of undeclared ethnicity. The ward provided an environment for strict control of food consumption, physical activity, and data collection. Each subject was provided a private bedroom with cable television, a radio with a wake-up alarm, an intercom to a 24-h central nurse station, a telephone, and a semiprivate bathroom. The activity areas and nurse station were adjacent to the private bedrooms. The subjects were allowed to leave the immediate living or dining areas, or facility, only when accompanied by a chaperone to ensure compliance with the study protocols. Meal consumption was observed by specially trained dietary staff, and irregularities were recorded. The subjects agreed to use only personal care products in the amounts approved by the principal investigators to limit and standardize extraneous chemical exposure. Subjects were not allowed to use tobacco, medicinal marijuana, or illegal drugs or to consume alcohol (except for specified ethanol tolerance tests). For most studies, individually prescribed physical activity was performed multiple times per week to maintain initial body composition and physical work capacity.

Diet

Composition

Basal diets were composed of ordinary Western foods, which were sometimes supplemented with experimental foods (eg, fructose cornbread, egg white drinks, and casein biscuits), and were fed as a 6-d (study 1) or 3-d (studies 2–19) menu rotation to provide variety but in a manner that ensured that the variations in nutrient intake were not consequential. Standard temperatures and cooking times were adhered to for each recipe in the menu cycles. Salt, pepper, coffee, and tea were served in constant amounts, selected by each subject, throughout the individual studies. The limited menus were supplemented as needed with some nutrients in constant amounts to maintain nutritional adequacy. Dietary iron (as ferrous gluconate) was provided in excess of the recommended dietary allowance to mitigate the decline in iron status as a result of phlebotomy during the studies.

Preparation and use

The subjects consumed only and all foods, beverages (including water), and vitamin, mineral, or other supplements provided by the center. The minimum length of any dietary period for any study was 18 d. Whenever possible, the food was purchased in single lots sufficient to last for several months to ensure minimal variation in food types. All the food was weighed with a 1% rounding error during preparation in the metabolic kitchen and was consumed completely by the subjects with the aid of spatulas and rinse bottles. Deionized water was consumed ad libitum. The

TABLE 1
Characteristics of the metabolic studies available for cross-sectional statistical analysis[1]

Study no., study design, and reference[2]	Study period	Sex	All subjects	Qualified subjects[3]
1. Plant fiber: bioavailability of minerals in food (7)	May 1976–Feb 1982	M		
Age (y)			25.6 ± 8.8 (18.0–52.0)[4]	26.5 ± 9.1 (19.0–52.0)
Weight (kg)			78.5 ± 15.2 (58.9–135.8)	78.0 ± 12.5 (58.9–107.9)
Height (cm)			177.1 ± 7.6 (152.8–193.4)	175.5 ± 5.2 (166.3–182.0)
n			29	13
2. Dietary fiber: stool output (8)	May 1977–Dec 1980	M		
Age (y)			29.6 ± 11.1 (19.0–64.0)	33.9 ± 14.6 (19.0–64.0)
Weight (kg)			77.4 ± 12.3 (60.3–107.9)	79.2 ± 15.2 (62.7–107.9)
Height (cm)			176.4 ± 9.3 (152.9–194.7)	173.2 ± 10.8 (152.9–194.0)
n			28	9
3. Physical performance and carbohydrate and lipid metabolism (9)	Sep 1980–Dec 1980	M		
Age (y)			23.7 ± 6.4 (20.0–31.0)	25.5 ± 7.8 (20.0–31.0)
Weight (kg)			70.6 ± 5.1 (67.3–76.4)	67.7 ± 0.5 (67.3–68.0)
Height (cm)			—[5]	—[5]
n			3	2
4. Browned and unbrowned corn products: bioavailability of zinc (10)	Jan 1981–Apr 1981	M		
Age (y)			35.3 ± 20.1 (22.0–65.0)	35.3 ± 20.1 (22.0–65.0)
Weight (kg)			73.9 ± 6.9 (68.0–83.7)	73.9 ± 6.9 (68.0–83.7)
Height (cm)			176.4 ± 9.4 (165.0–186.4)	176.4 ± 9.4 (165.0–186.4)
n			4	4
5. Intrinsically and extrinsically labeled meals: ^{65}Cu absorption (11)	Oct 1982–Dec 1984	M		
Age (y)			27.2 ± 9.1 (19.0–49.0)	28.1 ± 9.7 (19.0–49.0)
Weight (kg)			67.9 ± 6.4 (57.5–80.1)	67.0 ± 6.6 (57.5–80.1)
Height (cm)			174.7 ± 4.6 (167.7–184.0)	173.6 ± 3.8 (167.7–179.3)
n			11	9
6. Fat, vitamin E, and zinc intakes: copper and iron absorption and retention[6]	July 1982–June 1983	M		
Age (y)			28.3 ± 5.7 (22.0–37.0)	28.0 ± 6.1 (22.0–37.0)
Weight (kg)			76.2 ± 14.5 (48.3–91.0)	77.3 ± 15.6 (48.3–91.0)
Height (cm)			177.4 ± 7.3 (165.4–189.2)	177.3 ± 8.0 (165.4–189.2)
n			7	6
7. Dietary Maillard products: iron and zinc absorption and retention[6]	Aug 1983–May 1984	M		
Age (y)			26.1 ± 6.1 (20.0–39.0)	26.1 ± 6.1 (20.0–39.0)
Weight (kg)			73.4 ± 13.5 (51.4–91.4)	73.4 ± 13.5 (51.4–91.4)
Height (cm)			175.4 ± 5.6 (165.2–186.6)	175.4 ± 5.6 (165.2–186.6)
n			9	9
8. Folic acid supplements: zinc and iron absorption (12)	Jan 1984–July 1984	M		
Age (y)			29.1 ± 5.3 (19.0–36.0)	29.1 ± 5.3 (19.0–36.0)
Weight (kg)			85.9 ± 22.1 (64.8–134.7)	85.9 ± 22.1 (64.8–134.7)
Height (cm)			180.6 ± 10.3 (161.7–193.6)	180.6 ± 10.3 (161.7–193.6)
n			8	8
9. Copper and sucrose interactions[6]	May 1984–Dec 1984	M		
Age (y)			26.6 ± 4.5 (21.0–32.0)	26.6 ± 4.5 (21.0–32.0)
Weight (kg)			70.8 ± 3.4 (67.3–75.8)	70.8 ± 3.4 (67.3–75.8)
Height (cm)			180.8 ± 7.1 (170.1–189.9)	180.8 ± 7.1 (170.1–189.9)
n			7	7
10. Intrinsically and extrinsically labeled meat, liver, and peanut and sunflower butter: ^{65}Cu absorption (13)	Jan 1985–July 1985	F		
Age (y)			57.6 ± 5.3 (49.0–66.0)	57.6 ± 5.3 (49.0–66.0)
Weight (kg)			75.1 ± 12.4 (57.4–90.8)	75.1 ± 12.4 (57.4–90.8)
Height (cm)			165.3 ± 6.4 (155.4–172.5)	165.3 ± 6.4 (155.4–172.5)
n			7	7
11. Ascorbic acid and copper intakes: indicators of copper nutriture (14)	July 1985–June 1986	F		
Age (y)			26.8 ± 4.8 (20.0–37.0)	25.1 ± 3.5 (20.0–29.0)
Weight (kg)			61.3 ± 10.0 (46.0–76.0)	65.5 ± 9.9 (48.2–76.0)
Height (cm)			163.0 ± 4.0 (154.6–168.5)	163.0 ± 5.2 (154.6–168.5)
n			13	7

(*Continued*)

TABLE 1 (*Continued*)

Study no., study design, and reference[2]	Study period	Sex	All subjects	Qualified subjects[3]
12. Short-term and long-term variability of nutritional status indexes (15)	July 1985–Dec 1985	F		
Age (y)			30.6 ± 8.0 (23.0–44.0)	30.6 ± 8.0 (23.0–44.0)
Weight (kg)			86.9 ± 7.5 (76.8–92.7)	86.9 ± 7.5 (76.8–92.7)
Height (cm)			170.2 ± 2.2 (168.1–172.9)	170.2 ± 2.2 (168.1–172.9)
n			5	5
13. Weight loss: menstrual symptomatology (16)[7]	July 1990–Dec 1990	F		
Age (y)			26.8 ± 4.4 (21.0–38.0)	26.8 ± 4.4 (21.0–38.0)
Weight (kg)			93.1 ± 13.8 (75.2–115.9)	93.1 ± 13.8 (75.2–115.9)
Height (cm)			162.9 ± 6.3 (155.0–176.5)	162.9 ± 6.3 (155.0–176.5)
n			14	14
14. Oxidant stress by dietary factors: copper status indicators (17)	Jan 1991–Aug 1991	M		
Age (y)			31.0 ± 4.8 (24.0–37.0)	31.0 ± 4.8 (24.0–37.0)
Weight (kg)			82.8 ± 7.5 (73.0–97.7)	82.8 ± 7.5 (73.0–97.7)
Height (cm)			181.4 ± 7.2 (177.0–197.1)	181.4 ± 7.2 (177.0–197.1)
n			7	7
15. Meat consumption: zinc absorption and iron status (18)	July 1992–Dec 1992	F		
Age (y)			62.5 ± 6.2 (51.0–70.0)	62.5 ± 6.2 (51.0–70.0)
Weight (kg)			68.3 ± 10.8 (52.7–88.5)	68.3 ± 10.8 (52.7–88.5)
Height (cm)			159.7 ± 6.2 (145.1–172.7)	159.7 ± 6.2 (145.1–172.7)
n			14	14
16. Copper intakes: copper status indicators (19)	Jan 1993–July 1993	F		
Age (y)			62.5 ± 7.4 (49.0–75.0)	63.3 ± 7.2 (49.0–75.0)
Weight (kg)			67.2 ± 10.4 (45.6–83.1)	68.1 ± 10.3 (45.6–83.1)
Height (cm)			158.2 ± 6.1 (148.5–166.9)	158.9 ± 5.8 (148.5–166.9)
n			13	12
17. Weight loss: regional body composition (20)[7]	Jan 1994–June 1994	F		
Age (y)			29.6 ± 4.2 (25.0–38.0)	28.3 ± 3.4 (25.0–33.0)
Weight (kg)			100.3 ± 16.9 (78.0–132.5)	102.8 ± 21.9 (78.4–132.5)
Height (cm)			167.8 ± 6.4 (159.3–182.5)	170.5 ± 8.0 (160.2–182.5)
n			12	6
18. Fructose and magnesium intakes: macromineral homeostasis (21)	July 1994–Dec 1994	M		
Age (y)			30.4 ± 5.5 (22.0–40.0)	30.4 ± 5.5 (22.0–40.0)
Weight (kg)			78.9 ± 13.1 (53.4–94.8)	78.9 ± 13.1 (53.4–94.8)
Height (cm)			178.2 ± 9.0 (164.0–199.0)	178.2 ± 9.0 (164.0–199.0)
n			14	14
19. Magnesium and copper intakes: magnesium status indicators (22)	Jan 1995–Dec 1995	F		
Age (y)			63.8 ± 8.6 (47.0–78.0)	65.0 ± 7.2 (50.0–74.0)
Weight (kg)			67.4 ± 11.5 (50.1–89.9)	63.0 ± 11.2 (50.4–82.1)
Height (cm)			160.1 ± 6.0 (149.2–176.0)	161.0 ± 7.8 (149.9–176.0)
n			25	8

[1] Metabolic studies done at the US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND, between 1976 and 1995 measured individual dietary calcium intake and fecal and urinary calcium output.

[2] For the study design, the experimental variable or variables are given, and the primary outcome measure or measures follow the colon. The reference number is in parentheses.

[3] Data from a specific dietary period for a person were excluded when the intake of copper, iron, magnesium, phosphorus, or zinc fell below the respective Estimated Average Requirements or exceeded the respective 99th percentiles of usual intake from the 1994 Continuing Survey of Food Intakes by Individuals (for Fe, above the upper limit; 4, 25) to avoid confounding the results with concurrent nutritional stress. To maximize consistency in data across persons, balance periods <6 or >12 d in length were eliminated. To meet the design criteria suggested by the Food and Nutrition Board (4), the minimum acceptable dietary adaptation period was 12 d.

[4] $\bar{x}$ ± SD; range in parentheses (all such values).

[5] Height data were not available.

[6] Unpublished data obtained during the spans listed under the column entitled "Study period" (study 6: Harold H Sandstead; study 7: Phyllis E Johnson; study 9: Leslie M Klevay).

[7] Study used data from maintenance diets only.

initial energy requirement for each subject was determined by using the Harris and Benedict equation (23) and by adding a uniform amount (between 50% and 70%) of basal energy expenditure for normal physical activity. Except for the weight-loss studies (studies 13 and 17), energy intake was adjusted in standardized increments [typically 0.84 MJ (200 kcal)] during the course of each experiment

to maintain body weight (measured daily) within 2% of admission weight. The present study used balance data collected only during the initial equilibration dietary periods of the weight-loss experiments.

Dietary calcium

To ensure that the estimate of the calcium requirement was based on the forms of dietary calcium typically consumed, only studies that used diets not supplemented with calcium were selected for statistical analysis.

Calcium balance method

Dietary analysis

Balance data from the final 6–12 d of each dietary period of each study were analyzed. A duplicate diet for each subject in studies 1-10 and one representative duplicate diet supplying 8.4 MJ (2000 kcal) for all subjects in studies 11–19 were prepared daily for analysis. For all the studies, the daily duplicate diets were blended, and aliquots (6% of total weight) of the daily meals were mixed well and made into 6- or 7-d composites before being frozen. Aliquots of all individualized foods (eg, standardized amounts of additional salt), prescribed dietary supplements, and transient medications were analyzed for mineral content, and the mineral contributions were included as part of dietary calcium intakes.

Urinary and fecal analyses

Total urine was collected with the use of polypropylene funnels into 4-L polypropylene containers (6 mL of 6N HCl as "Baker Technical" grade was added to prevent bacterial growth; JT Baker Inc, Phillipsburg, NJ). Fecal output, excluding remains left on toilet paper, was collected directly in plastic bags throughout each study with precaution to avoid trace mineral contamination. All excreta were cooled immediately after collection in a double-doored refrigerator that provided discreet sample transfer to technical staff. The final 6- or 7-d urine composites of each dietary period for each subject were prepared by combining proportional aliquots of daily urine collections and freezing them until analyzed. Weighed fecal specimens were frozen, then lyophilized, and finally combined in toto in a plastic bag to prepare 6- or 7-d composites for each subject and were subsequently pulverized by use of a rolling pin and mixed by being shaken by hand.

Exercise

In all the studies, each subject was required to exercise ≥15 min at 50% maximum work capacity on an ergocycle 3 times/wk. Additional exercise was prescribed as needed to maintain body weight within 2% of initial weight. Voluntary walking regimens did not affect mandatory exercise prescriptions.

Calcium balance determination

Dietary and fecal sample digestion and analyses

Aliquots of the diet and fecal composites were digested with perchloric and nitric acids in glass beakers. The calcium contents of the dietary, urinary, and fecal digestates were determined by flame atomic absorption spectrophotometry (studies 1–4) or by inductively coupled plasma emission spectroscopy (studies 5–19) with aqueous calibration standards. Urine samples were diluted in 0.5% lanthanum chloride before calcium measurement by flame atomic absorption spectrophotometry to mask interferences. The methodologic precision and accuracy of the digestion and analytic procedures were evaluated by concurrent analysis of the National Institute of Standards and Technology bovine liver standards (standard reference materials no. 1577), pool samples, and replicate samples containing added calcium.

Calculation of calcium balance

Whole-body surface losses of calcium were determined (unpublished data) for young men (1989) (24) and young overweight women (1990) (16) in 2 of the metabolic studies. Each subject showered, then put on a cotton suit of long underwear and a protective covering over briefs and socks, all provided by the metabolic unit. After 48 h, the suits were removed, and the subjects stood in a plastic tub and showered with warmed deionized water. Shaving or application of any skin care product other than the wash soap provided by the staff was not allowed for 4 d before or during the sweat test.

The last balance periods (≤2) were selected from each available dietary period to provide 1–9 observations per subject. Calcium output was calculated as the sum of fecal and urinary losses. Calcium balance was calculated by differences between dietary intake and fecal and urinary losses. Calcium balance and output calculations did not include various negligible excretory calcium losses based on analytic determinations (unpublished data): whole-body surface [3.0 mg Ca/d for young men (data collected in 1989); 16.7 mg Ca/d for young overweight women (data collected in 1990)]; semen (0.8 ± 0.4 mg Ca/d; data collected in 1989); menses (0.005 ± 0.004 mg Ca/menstrual day; data collected in 1987, 1990, and 1994); and phlebotomy (5.3 ± 0.8 mg Ca/blood drawing; data collected in 1992, 1995, 1996, and 1999).

Data management and statistical analysis

Confidentiality considerations

For each original study, a password-secure, confidential computer file maintained the linkage between subject name and identification number, with access limited to select center staff members. This linkage was separate from research data files and was kept for several reasons: to provide subjects with individual study results and to provide governmental and institutional auditors with access as required by law. For the present statistical analysis, the linkage between preexisting data files and subjects was broken by the following method. The database for the original studies consisted of multiple data files, each of which was keyed by using a unique individual identification number. A separate file was created that consisted only of records with current identification numbers along with new, randomly generated identification numbers. Subsequently, the relevant data files in the preexisting database were copied into a new database; as the file was copied, the original identification number was replaced by the randomly generated identification number. Subject names and birth dates were not copied to the new database. After the new database was created, the file containing the existing identification numbers and the randomly generated identification numbers was deleted permanently. No hard copy of this file was ever generated. In summary, all linkage was broken between the preexisting data and the original identification numbers with no possibility to associate any original data with a specific subject.

Sample exclusion criteria

Data from a specific dietary period for an individual were excluded when intakes of magnesium, copper, iron, phosphorus, or zinc fell below the respective EAR or exceeded the respective 99th percentiles of usual intakes from the 1994 Continuing Survey of Food Intakes by Individuals (for iron, above the upper limit; 4, 25) to avoid confounding the results with concurrent nutritional stress. To maximize the consistency in the data across individuals, balance periods <6 or >12 d in length were eliminated. To meet the design criteria suggested by the Food and Nutrition Board (4), the minimum dietary adaptation period was 12 d (median: 31 d; maximum: 109 d).

Model

For calcium, the respective relations between mineral intake [in mg/d (model A), in $mg \cdot kg$ body $wt^{-1} \cdot d^{-1}$ (model B), or in $mg \cdot kcal^{-1} \cdot d^{-1}$ (model C)] and mineral output (fecal + urinary excretion) were investigated by using the following linear mixed-effect model (26):

$$Y_{ij} = \alpha + \beta X_{ij} + a_i^* + b_i^* X_{ij} + e_{ij} \qquad (1)$$

where Y_{ij} is the jth calcium output measurement on the ith subject, X_{ij} is the jth calcium intake measurement on the ith subject, $i = 1,..., 154$ subjects, $j = 1,..., n_i$ values, α and β are fixed effects, a_i^* and b_i^* are random variables that are assumed to follow a multivariate normal distribution with mean zero and variance-covariance matrix

$$\Sigma = \begin{pmatrix} \sigma_a^2 & \sigma_{ab} \\ \sigma_{ab} & \sigma_b^2 \end{pmatrix} \qquad (2)$$

and e_{ij} are the error effects that are assumed to be normally distributed random variables with mean zero and variance σ_e^2. Thus, the fixed effects part of the model is $\alpha + \beta X_{ij}$, whereas the random-effects part of the model is $a_i^* + b_i^* X_{ij} + e_{ij}$. This model is appropriate when there are multiple observations from independent subjects and the regression model for each subject can be assumed to be a random deviation from some overall population regression model. PROC MIXED in SAS (version 9.1; SAS Institute, Cary, NC) was used to fit all models. An unstructured variance-covariance matrix, Σ, was specified for the slopes and intercepts. As specified in Equations *1*, both intercepts and slopes are considered random effects. To test whether random intercepts and slopes were necessary, additional models were fitted to allow for only random intercepts and only random slopes. Akaike's Information Criterion was used to compare models. Some models did not converge when both slopes and intercepts were considered random effects. For those models that did converge, Akaike's Information Criterion indicated that the models that treated the slopes as random effects and treated the intercept as a fixed effect provided the best fit; thus, this reduced parameterization was used for all models.

Fixed coefficients were added to the models to allow for separate intercepts and slopes for men and women to determine whether sex differences existed in the requirement estimates. The model used was the following:

$$Y_{ij} = \alpha + \beta_1 X_{ij} + \beta_2 G_i + \beta_3 X_{ij} G_{i_i} + b_i^* X_{ij} + e_{ij} \qquad (3)$$

where $G_i = 1$ if subject i was male or $G_i = 0$ if subject i was female. To test whether there was an age effect on calcium requirements, age was added to the model as follows:

$$Y_{ij} = \alpha + \beta_1 X_{ij} + \beta_2 A_i + \beta_3 X_{ij} A_i + b_i^* X_{ij} + e_{ij} \qquad (4)$$

where $A_i = 1$ if subject i was >50 y of age; and $A_i = 0$ if subject i was aged ≤50 y. Age was dichotomized rather than included as a continuous variable because the distribution of ages was bimodal in this sample.

To assist in the assessment of the fit of the models, several influence diagnostics within PROC MIXED were calculated and plotted. One subject was identified by all methods as an outlier having an unacceptably large influence on the parameter estimates and the covariance estimates. That subject was subsequently removed from all statistical analyses.

The 95% CIs (27) and 95% prediction intervals (28, 29) were calculated and graphed for the final models. The calcium requirements were defined as the point at which calcium intake equaled calcium output. The 95% prediction interval around this point was obtained by considering the estimation to be a calibration problem (ie, the inverse prediction of calcium intake given calcium output) and calculating the prediction intervals by using methods for random coefficient models (29).

RESULTS

A total of 373 observations from 155 subjects were available for statistical analysis. Daily intakes of calcium ranged between 415 and 1740 mg. To facilitate the application of the findings to the general population, the data were examined by 3 statistical models: model A (expressed as mg/d), model B (expressed as $mg \cdot kg$ body $wt^{-1} \cdot d^{-1}$), and model C (expressed as $mg \cdot kcal^{-1} \cdot d^{-1}$).

Calcium intake compared with output: statistical model A

Calcium output (Y) increased linearly with increases in calcium intake (C) when intake was expressed as mg/d ($P = 0.0001$; **Figure 1**). Calcium intake equaled calcium output (crossed line of unity; neutral balance) at intakes of 741 mg/d ($Y = 148.3 + 0.80C$; 95% PI: 507, 1035 mg/d). The relation between calcium output and intake, expressed as mg/d, was not sex dependent ($P = 0.5$). After adjustment for calcium intake, age did not affect calcium output within the age range tested ($P = 0.31$).

Calcium intake compared with output: statistical model B

Calcium output increased linearly with increases in calcium intake expressed as $mg \cdot kg$ body $wt^{-1} \cdot d^{-1}$ ($P = 0.0001$; **Figure 2**). Accordingly, calcium intake equaled calcium output at 9.39 $mg \cdot kg$ body $wt^{-1} \cdot d^{-1}$ ($Y = 1.44 + 0.85C$; 95% PI: 6.36, 12.94 $mg \cdot kg$ body $wt^{-1} \cdot d^{-1}$). Sex did not affect the relation between calcium intake and output ($P = 0.14$) when the relation was expressed as $mg \cdot kg$ body $wt^{-1} \cdot d^{-1}$. Also, after adjustment for calcium intake, the effect of age was not statistically significant within the age range tested ($P = 0.1$).

Calcium intake compared with output: statistical model C

Calcium output increased linearly with increases in calcium intake ($P = 0.0001$) when both were expressed as $mg \cdot kcal^{-1} \cdot d^{-1}$ (**Figure 3**). Across all subjects, calcium intake equaled calcium
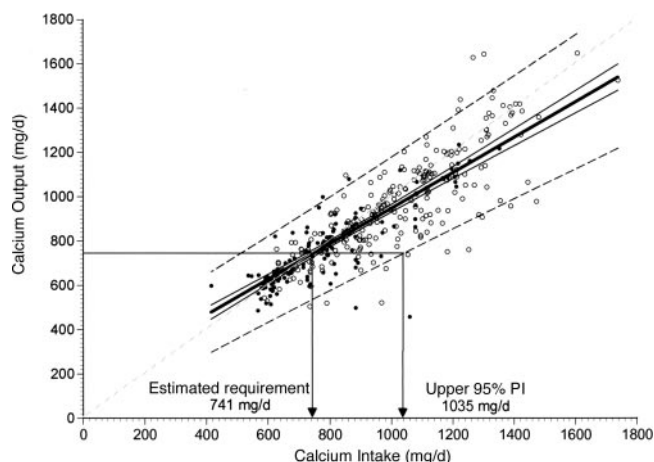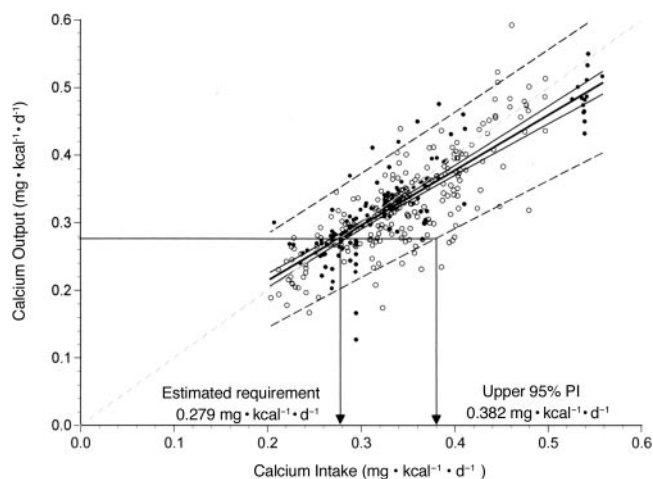
FIGURE 1. Relation between calcium output (fecal calcium + urinary calcium excretion) and calcium intake, both expressed as mg/d, for women [●; $n = 73$; weight: 77.1 ± 18.5 kg; age: 47.0 ± 18.5 y (range: 20–75 y)] and men [○; $n = 81$; weight: 76.6 ± 12.6 kg; age: 28.2 ± 7.7 y (range: 19–64 y)] who participated in tightly controlled feeding studies conducted in a metabolic unit (373 observations). Data were modeled by using a linear mixed-effect model. Calcium output (urinary + fecal) increased linearly with increases in calcium intake ($P = 0.0001$). Across all subjects, neutral calcium balance (defined as calcium output Y equal to calcium intake C) was maintained at calcium intakes of 741 mg/d ($Y = 148.29 + 0.80C$). Solid thin lines indicate 95% CIs around the regression line. Dotted lines indicate the 95% prediction interval.

output at 0.279 mg · kcal^{-1} · d^{-1} ($Y = 0.051 + 0.816C$; 95% PI: 0.194, 0.382 mg · kcal^{-1} · d^{-1}). Age was a significant predictor of output within the age range tested ($P = 0.03$) after adjustment for calcium intake. Calcium intake equaled calcium output for persons aged ≤50 y at 0.25 mg · kcal^{-1} · d^{-1} ($Y = 0.034 + 0.861C$) and at 0.33 mg · kcal^{-1} · d^{-1} ($Y = 0.089 + 0.729C$) for

FIGURE 2. Relation between calcium output (fecal calcium + urinary calcium excretion) and calcium intake, both expressed as mg · kg body wt^{-1} · d^{-1}, for women [●; $n = 73$; weight: 77.1 ± 18.5 kg; age: 47.0 ± 18.5 y (range: 20–75 y)] and men [○; $n = 81$; weight: 76.6 ± 12.6 kg; age: 28.2 ± 7.7 y (range: 19–64 y)] who participated in tightly controlled feeding studies conducted in a metabolic unit (373 observations). Data were modeled by using a linear mixed-effect model. Calcium output (urinary + fecal) increased linearly with increases in calcium intake ($P = 0.0001$). Across all subjects, neutral calcium balance (defined as calcium output Y equal to calcium intake C) was maintained at calcium intakes of 9.39 mg · kg body wt^{-1} · d^{-1} ($Y = 1.44 + 0.85C$). Solid thin lines indicate 95% CIs around the regression line. Dotted lines indicate the 95% prediction interval.

FIGURE 3. Relation between calcium output (fecal calcium + urinary calcium excretion) and calcium intake, both expressed as mg · kcal^{-1} · d^{-1}, for women [●; $n = 73$; weight: 77.1 ± 18.5 kg; age: 47.0 ± 18.5 y (range: 20–75 y)] and men [○; $n = 81$; weight: 76.6 ± 12.6 kg; age: 28.2 ± 7.7 y (range: 19–64 y)] who participated in tightly controlled feeding studies conducted in a metabolic unit (373 observations). Data were modeled by using a linear mixed-effect model. Calcium output (urinary + fecal) increased linearly with increases in calcium intake ($P = 0.0001$). Across all subjects, neutral calcium balance (defined as calcium output Y equal to calcium intake C) was maintained at calcium intakes of 0.279 mg · kcal^{-1} · d^{-1} ($Y = 0.051 + 0.816 C$). Solid thin lines indicate 95% CIs around the regression line. Dotted lines indicate the 95% prediction interval.

persons aged >50 y. Sex did not affect the relation between calcium intake and output ($P = 0.89$) when the relation was expressed as mg · kcal^{-1} · d^{-1}.

Indicators of calcium homeostasis

Mean apparent calcium absorption, expressed as a percentage of calcium intake, was 24.9 ± 12.4% and increased linearly with calcium intake ($P = 0.006$). Apparent calcium absorption was not significantly different ($P = 0.07$) between women <50 y of age (29.5 ± 17.0%) and women ≥50 y of age (23.3 ± 11.2%). Mean calcium intake for women aged ≤50 or >50 y was 821 ± 108 and 847 ± 232 mg/d, respectively. Mean calcium intake for men aged ≤50 or >50 y was 992 ± 207 and 836 ± 40 mg/d, respectively. The percentage of calcium intake lost in the urine and feces was 21.8 ± 7.7% and 75.1 ± 1.4%, respectively. Serum calcium concentrations (median: 2.35 mmol/L; 229 observations; 108 subjects) were not significantly affected by time of year ($P = 0.09$).

Subjects ranged between 19 and 75 y of age (**Figure 4**). To explore further the possible effects of age, calcium balance was modeled by using age as the predictor. No significant relation between calcium balance (expressed as mg/d) and age was indicated ($P = 0.4$).

DISCUSSION

The present study expands the calcium balance data needed to generate better estimates of the adult calcium requirement. As defined by the Food and Nutrition Board (4), useful balance studies for estimating the calcium requirement include subjects who consume a wide range of calcium intakes, include a minimum equilibration period of 7 d with the experimental diet before calcium balance is assessed, and, when possible, include only
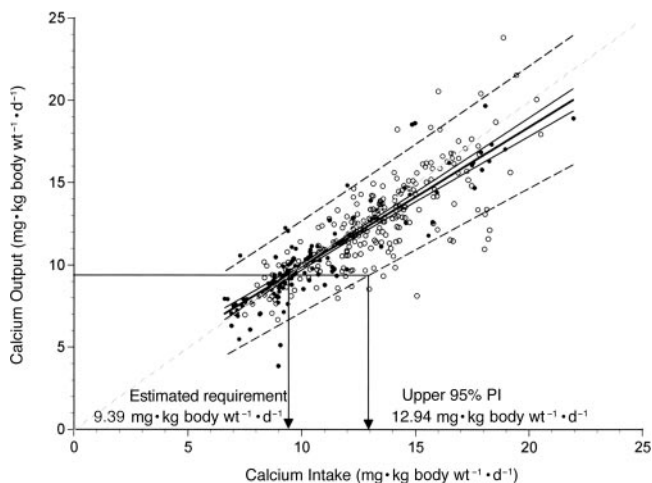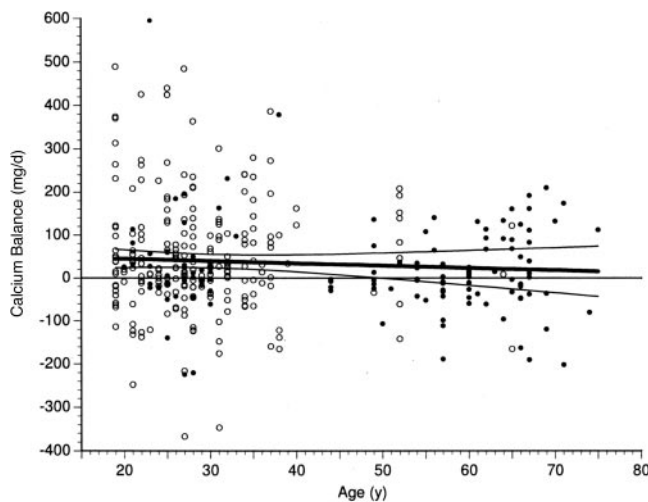
FIGURE 4. Relation between calcium balance (expressed as mg/d) and age for women [●; $n = 73$; weight: 77.1 ± 18.5 kg; age: 47.0 ± 18.5 y (range: 20–75 y)] and men [○; $n = 81$; weight: 76.6 ± 12.6 kg; age: 28.2 ± 7.7 y (range: 19–64 y)] who participated in tightly controlled feeding studies conducted in a metabolic unit (373 observations). Data were modeled by using a linear mixed-effect model. There was no significant relation between balance and age ($P = 0.4$). Solid thin lines indicate 95% CIs around the regression line.

subjects who were consuming their usual calcium intakes. The present study exploited the extensive metabolic data collected from a series of studies in which only healthy subjects participated, calcium intakes below and near the presumed required amounts were included, adequate dietary adaptation was ensured by examining only dietary periods ≥ 18 d, the mineral content of the drinking water was analyzed, food and beverages were prepared by professional staff for delivery within 1% weighing error, duplicate diets were prepared by professional staff, food consumption was quantitative and was carried out under visual supervision, fecal and urine collections were continuous to ensure familiarity with the procedure, and samples were analyzed by use of state-of-the-art technology.

The diets and environments used by the metabolic studies were a reasonable approximation of the free-living environment. The amount and type of exercise (swimming, walking, and stationary biking) were prescribed per individual and therefore varied considerably between individuals. All diets were constructed in a manner that resulted in a high degree of compositional heterogeneity across meals and studies. Mean ($\pm$SEM) calculated intakes of phosphorus (1100 ± 43 mg/2000 kcal) and fiber (11.2 ± 0.8 g/2000 kcal) were similar to respective national mean ($\pm$SEM) intakes of phosphorus (1222 ± 12.9 mg/d; 4) and fiber (15.2 ± 0.1 g/d) for all individuals (including pregnant and lactating women). Because calcium supplementation may differentially affect calcium metabolism and bias the results in favor of the experimental hypothesis that calcium balance is lower than are current estimates, data from the studies that used calcium supplementation (as calcium gluconate, calcium carbonate, calcium lactate, calcium citrate, or dicalcium phosphate) were excluded. The estimate of the amount of calcium intake needed to maintain neutral calcium balance was higher when calculated with data from studies that did not use calcium supplementation than when calculated with data from calcium supplementation studies (752 compared with 533 mg Ca/d; $P = 0.0001$).

Calcium homeostasis

The data indicate tight control of calcium homeostasis in the range of typical calcium intakes and far above the point at which calcium balance is neutral (741 mg/d; Figures 1-3). These characteristics of the statistical models indicate that calcium balance was highly resistant to a change in calcium intake across a broad range of typical dietary calcium intakes (415-1740 mg/d; between the $\approx$25th and >99th percentiles of typical calcium intake for all female children and adults aged ≥ 9 y). In other words, homeostatic mechanisms for calcium metabolism seem to be functional across a broad range of typical dietary calcium intakes to minimize calcium losses and accumulations. These mechanisms seem particularly active as indicated by the especially acute angle between the line of unity and the regression line. The experimental data do not support the hypothesis that phylogenic calcium conservation mechanisms failed to evolve because of an assumed early dietary calcium surplus with no selective advantage for calcium conservation (30).

Estimations of the average calcium requirement

The statistical model used in the present study predicted neutral calcium balance at calcium intakes of 741 mg/d for healthy individuals regardless of age or sex. This new presumptive value for an EAR for calcium is considerably lower than that of the existing AI. The AIs for calcium (sometimes considered equivalent to EARs for calcium; 31) are 1000 and 1200 mg/d for adults aged 19–50 and >51 y, respectively; the latter are $\approx$20% higher than are the mean calcium intakes of American males aged ≥ 9 y (925 mg/d). However, the mean calcium intake of American females aged ≥ 9 y (657 mg/d) falls below this value by 11% (4). In the general population, calcium intake decreases with age for both men and women (4). In the current study, the absence of an effect of age on calcium balance probably reflects higher mean calcium intakes than those of similar age-sex groups in the American population. Factors other than insufficient calcium intake also operate in the body during bone involution and lead to diminution in bone mass (31, 32).

There is no current recommended dietary allowance for calcium. Our data suggest a recommended dietary allowance of 1035 mg/d (the upper limit of the 95% prediction interval around the new estimation of the calcium requirement) for all adults. This level of calcium intake is not attained by >75% of the American population on average (4).

The current AI for calcium was set by modeling calcium retention compared with calcium intake by using the nonlinear Jackman model (33). We chose to model output rather than retention to eliminate possible confounding and reduction in the relative precision of parameter estimates caused by including intake as a component of the dependent variable (calcium retention). In the present analyses, the data did not show nonlinearity and therefore did not justify the use of a more complex nonlinear model. The coefficients of the AI model appear to be greatly influenced by data points above the 99th percentile of daily calcium intake. Data used in the model in the present study reflect typical calcium intake between the 5th and $\approx$95th percentiles for all male children and adults aged ≥ 9 y and between the $\approx$25th and >99th percentiles for all female children and adults aged ≥ 9 y.

This new estimate of the calcium requirement may resolve several discrepancies between current theory and observation.

For example, the new balance data also concur with the recognition that saturation of the active transport component of calcium absorption occurs at an intake of ≈500 mg/d (34). It seems unlikely that a physiologic mechanism that drives active calcium absorption in a dynamic range is concurrently unable to maintain normal calcium homeostasis. Also, most (35–39), but not all (40), studies with adults that indicate a positive influence of high dietary calcium in reducing the rate of bone remodeling were confounded by the presence of vitamin D as an experimental covariable. In the present study, only 2 of the metabolic diets (studies 18 and 19) were supplemented with vitamin D (200 μg/d) because of their exceptionally low calculated vitamin D content (0.5 μg/2000 kcal). The average (±SEM) calculated vitamin D content of all the metabolic diets was 2.9 ± 0.24 μg vitamin D/2000 kcal, which is very similar to the estimated median intake of vitamin D by free-living young women in a separate study (2.9 μg/d; 41). The new estimation is in line with the previous consideration that individuals with low, but nutritionally adequate, intakes of sodium and protein may have calcium requirements as low as 500 mg/d (30). The metabolic diets provided (mean ± SEM) 71.8 ± 3.0 g protein/2000 kcal and 2770 ± 135 mg sodium/2000 kcal [with additional individualized, standardized sodium consumption of up to 3100 mg/d (studies 1–10) or 0.775 mg/d (studies 11–19)]. The mean intakes of protein and sodium were similar to respective national mean (±SEM) intakes of protein (75.3 ± 0.4 g/d; 42) and sodium (3418 ± 31 mg/d; 43) for all individuals (including pregnant and lactating women). Thus, neutral calcium balance was achievable at calcium intakes of 741 mg/d for individuals consuming typical amounts of sodium and protein.

In conclusion, the calcium balance data reported in the present study represent the most extensive known pool of calcium balance data collected under tightly controlled experimental procedures. The new data may be useful in establishing an EAR for calcium that may be lower than previously considered.

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